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buffs e^me^mss. Blacks are therefore genetically buffs with an epistatic gene for the complete extension of black pigment.

3. Extension of black is incompletely epistatic over silver so that in fowls of the genotype E^me^mSs (male) or E^me^mS-(female) silver appears in certain parts of the plumage, producing a pattern like that of the Dark Brahma.

Collateral evidence indicates that the gene for extension of black pigment (or one with similar effects) is present in Barred Plymouth Rocks and White Plymouth Rocks (as a cryptomere); and that it is absent in Columbian and buff varieties and in Rhode Island Reds.

Hurst[3] was probably dealing with the same gene in his crosses between Black Hamburgs and Buff Cochins, since F_1 from this cross consisted of all black chicks, while in F_2 black and buff chicks occurred in the proportions of 88 black: 31 buff. In interpreting the results of this cross Morgan[4] states that either one or two pairs of factors may be involved; which is right "could only be determined by an F_2 ratio." Yet the F_2 ratio is given by Hurst (p. 138) and is surely a sufficiently close approach to a monohybrid ratio. The ratios obtained in our experiments agree throughout with a mono-factorial interpretation.

It is believed that this gene will be found to characterize many color varieties of fowls in which black, either as a self color or as a component of a pattern extends to all or nearly all of the plumage. Concerning its origin no direct evidence can be offered at present. Its occurrence as a discrete unit indicates, however, that its origin was discontinuous and that black varieties probably had their genesis in mutation rather than in selection of particolored types toward black.

L. C. DUNN

THE EFFECTS OF SO-CALLED CONJUGATION IN SHELLED RHIZOPODS[1]

THE phenomenon of conjugation in the Protozoa is regarded as the forerunner of sexual reproduction in the higher animals, in which there is a union of a sperm and an egg. This latter process is fundamental in the life of at least all the higher Metazoa. By this union the race is perpetuated and hereditary characters are intermingled. It would seem that studies of a similar process in the unicellular forms might throw light upon the basic relations of the sexual process to the life of protoplasm.

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[3] Hurst, C. C., 1905, Reports to the Evo. Comm., II, pp. 138–39.

[4] Morgan, T. H., 1919, Publ. Carnegie Inst. No. 285, p. 24.

[1] The experimental work on this problem was carried on in the Zoological Laboratory of the Johns Hopkins University. I wish to thank Dr. H. S. Jennings, of that institution, for suggesting the problem and for his aid in pursuing the investigations.

Various investigators have shown that conjugation is a relatively common occurrence among the more complex Protozoa, such as the Paramecium, and that hereditary characters are intermingled in this way. During conjugation two Paramecia fuse by their oral surfaces and there is an interchange of nuclear material between the individuals. The latter then separate and reproduction by division occurs. The races resulting from such divisions show the effects of modifications due to hereditary characters coming from both the conjugating individuals.

In the case of the more primitive Protozoa, the Amœba and other Rhizopods, not a great deal is known. It has been observed that sometimes two individuals unite, but it is uncertain whether or not true conjugation occurs with interchange of nuclear material and subsequent modification of the offspring. If such proves to be the case, we shall have shown that the phenomena of sex are found in the very lowest animals, and are of general fundamental importance in the life process.

The present investigation was undertaken in the effort to throw some light on this question. An attempt was made to test the matter by inducing conjugation between various individual Rhizopods and noting if any inherited differences arose from these unions. (A cytological study of the behavior of the nuclei of such individuals must be made before the evidence can be fully weighed.) A shelled Rhizopod, *Difflugia corona,* was used, since the shell exhibits marked characteristics which vary among the different races of the species. The ordinary shellless Amœba presents so few characters of a permanent nature that it is unsuited for a study such as this. The Difflugia is an Amœba which builds from microscopic sand grains a shell shaped much like an old-fashioned soap kettle with the legs represented by spines projecting from the rounded aboral surface. The animal lives inside this shell and thrusts its pseudopodia from the oral opening. It reproduces by division, as other Protozoa, half the body being extruded from the oral opening of the shell and a new shell being formed over the exposed part of the body, the two shells, old and new, lying mouth to mouth. The body then divides and the new individual moves away to lead a separate existence.

At times two Difflugias have been observed attached mouth to mouth and have been thought to be conjugating. When dividing the new shell is much lighter in color than the old and in the cases supposed to be conjugating both shells were of the same shade, that is both dark, so that the phenomenon did not seem to be that of division. It was this attachment or so-called conjugation which I endeavored to investigate.

It was found by Dr. Jennings (not published as far as I am aware) that two Difflugias could sometimes be made to attach themselves together by keeping them in a drop of distilled water for a few hours. At his suggestion, I used this method, endeavoring to hasten the process somewhat by pushing the individuals together by means of a fine glass rod. In several cases, the members of the pair became quite firmly united and remained so for some time. These pairs I then put into a drop of culture medium in the concavity of a hollow ground slide. In some cases such individuals never separated but died. In several instances they did separate and lived and began to reproduce by division.

Briefly, my procedure then was this. The number of spines varies in different races of Difflugia, sometimes averaging 2 or 3, sometimes as high as 5 or 6. The size of the shell also varies. I used the diameter across the widest part of the shell. I selected, for example, a small individual with a few spines and kept it under observation on a hollow ground slide in culture medium until it had produced an offspring by division. (The culture medium consisted of tap water containing the microscopic débris washed from the leaves of Elodea.) I then isolated the offspring and allowed it to produce a race, keeping each individual separate on a slide, and measuring and counting the number of spines of each. The original Difflugia I tried to mate, so to speak, with a large Difflugia with many spines, which had previously produced an offspring which I had isolated and allowed to found a race. If the large and small Difflugias became attached, supposedly conjugating, and were successfully separated, I then isolated each one on a slide and allowed each to start a line of progeny. I then had 4 lines going, one from each Difflugia before conjugation and one from each after conjugation with the other. The following diagrams may serve to make my meaning clear.

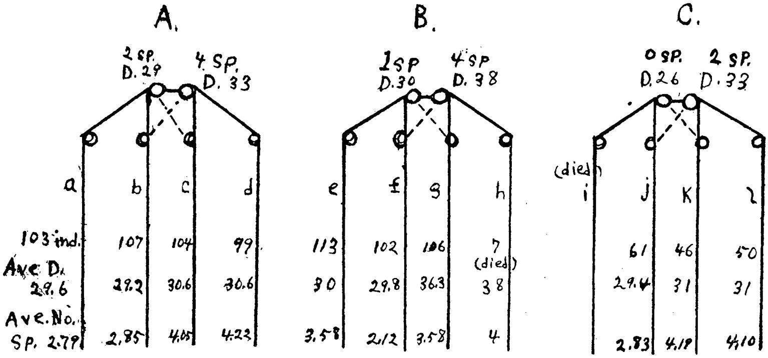

FIG. 1. Diagrams illustrating the manipulation of Difflugia in the experiments with so-called conjugation and the results attained in three cases. The two circles at the top of each diagram represent the individual Difflugias which became attached to each other. D., diameter- sp., spines. ind., individuals. Ave., average.

In each figure the circles at the top represent the Difflugia which were paired. For example, in *A* an individual with a diameter of 29 units founded a line *a* and then was paired with a Difflugia with 4 spines with a diameter of 33. This had previously started line *d*. After the separation of the Difflugias, they founded lines *b* and *c* respectively. The dotted cross lines represent the influence which might be expected to come from the other member of the pair if there were actual conjugation. Line *a* was carried on until 103 individuals were produced, the average number of spines being 2.79 and the diameter 29.6 units. Line *b*, started after the attachment of the progenitor to the other Difflugia, was carried for 107 individuals, with an average of 2.85 spines and a diameter of 29.2. The diagrams show the results gained from carrying out the lines in the other experiments. Unfortunately line *i* died before producing any individuals and line *h* produced only 7 before dying out.

By comparing the four lines in each experiment with each other, evidence may be gained relative to whether or not, during the supposed conjugation, the Difflugias exerted an effect upon each other sufficient to cause modification of the diameter and number of spines of the offspring in the direction of the line started by each before the attachment. That is, for example, line *b* should be more like line *d* than is line *a*, and line *c* should be more like *a* than is line *d*, and so on throughout the experiments.

A study of the data set forth in the diagrams will show that there is practically no modification of any line springing from a Difflugia, after the supposed conjugation, in the direction of the characters of the line founded by the other member of the pair before the union. Any slight changes seeming to show such modification are offset by just as marked variations away from that line. Comparing line *g* with *h*, it would seem that there is a marked modification of *g* in the direction of line *e*. It must be noted, however, that line *h* consists of only 7 individuals, so that the average is untrustworthy. In no other case is there any significant leaning toward the other line, although there are very slight tendencies in that way in the spine numbers in experiment A. Line *a* has 2.79 spines, *b* 2.85, *c* 4.05 and *d* 4.22. On the other hand, in experiment B the spine number in line *e* is 3.58 and in *f* only 2.12, to be compared with 4 in line *h*. It would be expected that line *f* would show a greater number of spines than line *e*. In experiment C line *k* shows a slight increase in spine number instead of the expected decrease. In general there are no apparent modifications of the offspring as the result of the pairing.

The experiments are open, of course, to the criticism that the attachment between the Difflugias was brought about by keeping the individuals in distilled water to bring them to the state of partial starvation which seems usually to be the forerunner of conjugation in the Protozoa. It is possible that under these somewhat unnatural conditions, the preliminary steps incident to conjugation would be inaugurated but that the process would stop before completion. In answer to this, I can only state that the Difflugias remained attached from 12 to 24 hours, thus allowing sufficient time for nuclear changes to have occurred. They became separated only when placed in cultures containing food.

To summarize then, as far as it is safe to base conclusions on the results of this limited number of experiments, it seems that the offspring of the Difflugias were not influenced by the attachment or so-called conjugation of the parents. From this fact it appears probable that this phenomenon of attachment sometimes observed in the shelled Rhizopods is not a true conjugation and that there is no interchange of nuclear material between the individuals taking part in it.

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